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Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L.

Ayesha Muzamil [a], Hafiz Muhammad Tahir [a,*], Aamir Ali [a], Muhammad Farooq Bhatti [b], Fariha Munir [a], Fatima Ijaz [a], Muhammad Adnan [a], Hooria Ashraf Khan [a], Kunza Abdul Qayyum [a]

[a] *Department of Zoology, Government College University, Lahore, Pakistan*
[b] *Sericulture Wing, Punjab Forestry, Wildlife and Fisheries Department, Ravi Road, Lahore, Pakistan*

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ABSTRACT

The demand for silk has been increasing day by day but the average silk production is not enough to meet its demand. In this study, we investigated the effect of amino acid supplemented mulberry feed on the biological and commercial traits of *Bombyx mori* L. (Lepidoptera; Bombycidae). The silkworm larvae at 5th instar stage were taken and fed with fresh and healthy mulberry leaves coated with Alanine, Glycine and Serine in fourteen different combinations. Results of the current study revealed that the average weight of silkworm larvae and the % ratio of silk gland to body weight on day 7 was significantly ($P<0.05$) higher in the group fed with amino acid fortified leaves as compared to the control. The commercial traits of larvae fed with amino acid fortified leaves also improved significantly. The larvae fed with Alanine (1 %) treated mulberry leaves showed the maximum cocoon weight, cocoon length, cocoon width, cocoon shell ratio and fibroin content as compared to the control group. It is evident from the results that the amino acid (particularly alanine) coated mulberry leaves have a positive effect on the commercial and biological traits of *Bombyx mori* (L.).

1. Introduction

Asia is the world's top silk producing region, contributing for 98 % of worldwide output [1]. Silkworm rearing is a profitable and successful commercial activity that needs little input and can improve the economic situation of rural people [2]. During last three years, the rise in silk production has not fulfilled the global demand for silk. This decrease in silk production is due to low-quality silk seed, unavailability of mulberry leaves, low price and poor quality of cocoons with fewer marketing facilities [3,4]. It is necessary to overcome these problems in order to promote the sericulture industry in Pakistan.

Silk is a natural, genetically encoded, long spun filament of fibrous protein, produced by various arthropods including Arachnids and Lepidoptera [5,6]. Silk protein from silkworm (*Bombyx mori* L.) is a highly utilized fiber because of its biocompatibility, biodegradability and elasticity [7]. It is a luxury natural fiber that can't be compared to cheap synthetic fibers (polyester and viscose) that are being used extensively in textiles [8,9].

Silkworm (*B. mori*) is a monophagous lepidopteron insect that is reared for silk production under a special controlled environment

* Corresponding author.
E-mail address: dr.hafiztahir@gcu.edu.pk (H.M. Tahir).

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[2]. Silkworm derives all essential nutrients from mulberry leaf for growth and development [[10–12]]. The growth and development of silkworm larvae is greatly influenced by the mulberry leaves quality [[13–15]]. The production of cocoons is also affected by different factors including environmental conditions, planting and pruning and soil characteristics that affect mulberry leaf quality [16]. The dietary and nutritional requirements of the silkworm including water, vitamins, carbohydrates, proteins, fats and ascorbic acid are met by digestion and assimilation of mulberry leaves [17,18]. Moreover, mulberry leaf proteins are directly responsible for over 70 % of the silk production by silkworms, subsequently affecting the overall cocoon production [[19–21]].

The silk quality and production can be enhanced by improving silkworm rearing as well as providing high quality mulberry leaves and feed supplements [22,23]. The artificial nutritional supplements of the silkworm diet include Vitamin C, vitamin B complex, minerals and juvenile hormones [24]. These supplementations not only provide the necessary nourishment for improved growth and development but also improve economic characteristics [23]. Recently, amino acid fortified mulberry leaves has been introduced in Sericulture industry to improve the economic and biological traits of silkworm (larval growth, cocoon shell size and weight of silkworm) and the quality of silk produced [[25–27]]. They can regulate and adjust the physiological function and metabolism of silkworms by joining active substances such as hormones and enzymes.

The present study has been undertaken to enhance the commercial and biological traits of silkworms by feed supplementation with amino acid (Alanine, Glycine and Serine) fortified mulberry leaves. The increase in silk production in Pakistan will not only help to improve silk productivity to meet local silk demand but also increase our silk exports which will have a direct impact on the economy of Pakistan.

2. Materials and methods

2.1. Silkworm rearing

The silkworm (*B. mori*) eggs of Bulgaria Race (Hybrid) were obtained from the Sericulture Wing; Forestry, Wildlife and Fisheries Department, Lahore. Silkworm eggs were incubated under standard light (12 h light: 12 h dark), optimum temperature (25–28 °C) and humidity (70–75 %) for 8 days in the Research Laboratory of Sericulture Wing; Forestry, Wildlife and Fisheries Department, Lahore (31° 36′ 28″ N, 74° 18′ 11″ E) [[28–32]]. The eggs were hatched after 8 days [33]. The rearing room and equipment were cleaned and disinfected with 2 % formalin and 0.05 % of detergent [34]. The disinfected room and equipment were made airtight for 24 h for effective results. After that, the room was kept open for 12 h to get rid of formalin vapors. Newly hatched larvae were kept in sanitized trays and fed with fresh tender chopped mulberry leaves till the fourth instar [5]. 1st 2nd 3rd and 4th instar larvae were fed for two, three and four times a day respectively. Fifth-instar larvae were selected for the experiment and fed five times a day.

2.2. Preparation of different concentrations of solutions

Three amino acids including Alanine (Sigma-Aldrich LOT#MKB5487V), Glycine (Sigma-Aldrich LOT#SLCG0879) and Serine (UNI-Chem S19510-3D) were purchased from the market. Specific amounts of amino acids (Alanine, Glycine and Serine) were added to distilled water and different concentrations were prepared to evaluate the individual and synergistic effect of amino acids (Table 1). Fresh, clean, healthy mulberry leaves were dipped into the respective amino acids solution for 5 min [35,36]. After that, the leaves were shade dried for 5 min for proper and even adsorption of amino acids.

2.3. Experimental design

The 5th instar silkworm larvae (n = 225) were tagged and divided into fifteen groups, each group consisted of 15 larvae that were kept in a separate cardboard box (30 cm × 30 cm x 5 cm). Group 1 was considered as a control group and fed with fresh mulberry leaves, while groups 2–15 were considered as experimental groups that were given amino acid coated mulberry leaves as their first

Table 1
Detail of preparation of different concentrations of solutions.

Groups	Concentrations	Preparation
Group 02	1 % Glycine	1 g Glycine +100 ml deionized water
Group 03	1 % Alanine	1 g Alanine +100 ml deionized water
Group 04	1 % Serine	1 g Serine +100 ml deionized water
Group 05	1 % Alanine + Glycine	0.5 g Alanine +0.5 g Glycine +100 ml deionized water
Group 06	1 % Alanine + Serine	0.5 g Alanine +0.5 g Serine +100 ml deionized water
Group 07	1 % Serine + Glycine	0.5 g Serine +0.5 g Glycine +100 ml deionized water
Group 08	1 % Alanine + Glycine + Serine	0.33 g Alanine +0.33 Glycine +0.34 g Serine +100 ml deionized water
Group 09	0.5 % Glycine	0.5 g Glycine +100 ml deionized water
Group 10	0.5 % Alanine	0.5 g Alanine +100 ml deionized water
Group 11	0.5 % Serine	0.5 g Serine +100 ml deionized water
Group 12	0.5 % Alanine + Glycine	0.25 g Alanine +0.25 g Glycine +100 ml deionized water
Group 13	0.5 % Alanine + Serine	0.25 g Alanine +0.25 g Serine +100 ml deionized water
Group 14	0.5 % Serine + Glycine	0.25 g Serine +0.25 g Glycine +100 ml deionized water
Group 15	0.5 % Alanine + Glycine + Serine	0.16 g Alanine +0.17 Glycine +0.17 g Serine +100 ml deionized water

meal each day while the other four meals were fresh mulberry chopped leaves [14]. Bed cleaning was carried out daily before their first meal. Fifth instar larvae, when fully matured, were handpicked and transferred to montages for spinning. The experiment was replicated thrice and 675 larvae belonging to 15 different groups were reared and fed with amino acids coated mulberry leaves at the 5th instar.

2.4. Estimation of biological traits

The weight of the larvae (n = 15) was observed for all 15 groups on a daily basis till the day before spinning. An increase in the larvae weight was observed and noted. To assess the possible changes in the weight of silk glands due to the amino acid feed supplementation, 05 out of 15 silkworms of each group in each replicate were dissected before spinning and silk glands were taken out. The weight of the silk gland was measured by a digital vacuum weighing balance.

2.5. Estimation of economic traits

After completion of the 5th instar stage, the un-dissected silkworms started cocoon spinning. After the completion of the spinning process of cocoons in all study groups, the cocoons were kept in bright sunlight to kill the moth inside and eliminate any moisture content. The silk production in the control group was recorded and compared with treated groups. The weight (g) of cocoons (n = 10) was measured by using an electronic weighing balance while the length and width (mm) of cocoons were recorded by Vernier Caliper.

The dried cocoons (n = 10) from each group were cut with a cutter to remove dead moths present inside the cocoon. To compare the silk production of control and other treatment groups, the shell weight and shell ratio were calculated with the given formula 1 and 2 respectively.

1. Shell weight = Cocoon weight-pupal weight [17].

   After that, the shell ratio of the cocoon was calculated using the given formula.

2. Shell ratio (%) = Shell Weight (gm) x 100

   Cocoon Weight (gm)
   [26,37].

2.6. Sericin/Fibroin content

The sericin and fibroin content was inspected by autoclaving small pieces of cocoon in a specific amount of water at 121 °C for 2 h [38,39]. After that, the sericin was filtered and fibroin remained as filtrate on filter paper. The filtrate was dried at 70 °C for 24 h. Fibroin content was equal to the dry weight of filtrate after autoclaving followed by degumming to remove sericin. While given formula (3) was used to evaluate sericin [40]:

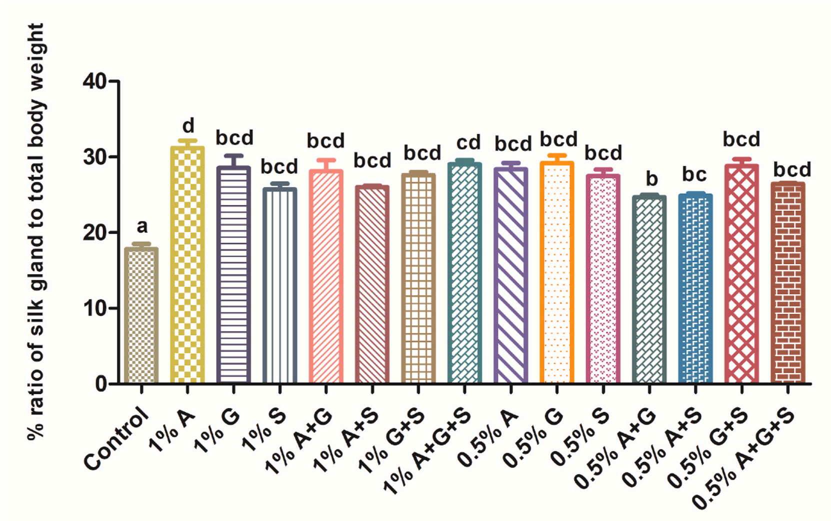

Fig. 1. Percentage ratio of silk gland weight to the total body weight of larvae in control and treatment groups. Groups with similar superscripts showed non-significant difference groups with different superscripts showed significant differences.

3. Sericin content (gm) = Initial dry weight of shell – Dry weight of shell/filtrate after filtration

2.7. Statistical analysis

For statistical analysis, the normality distribution of the variables was tested using the Shapiro-Wilk test. To analyze the results of the increase in larval weight Two-way repeated measure ANOVA was used followed by Tukey's test for the comparisons between study groups. One-way ANOVA was used to compare economic parameters (cocoon weight, cocoon length, cocoon width, percentage shell ratio, percentage fibroin content and percentage sericin content) of silkworms. P<0.05 was considered as significant value.

3. Results

3.1. Estimation of biological traits

The larval weights of silkworms (5th instar) increased gradually from day 1 to day 7 in experimental and control groups. There were significant differences among the larvae weights of control and experimental groups from day 2 to day 7. The increase in larval weight on day 6 was highest in the groups that were given amino acid fortified mulberry leaves. The average increase in weight was highest in the group fed with 1 % Alanine fortified mulberry leaves (0.665 g ± 0.013) as compared to all other treatment groups. The % ratio of silk gland to body weight in all treatment groups was higher as compared to the control group ($F_{14, 60} = 13.300$; P<0.001). The highest % ratio of silk gland to body weight was observed in the experimental group fed with 1 % Alanine treated mulberry leaves (31.208 ± 0.866) (Fig. 1).

3.2. Cocoon weight, width and length

Significant differences were recorded in the weight of cocoons between the control and experimental groups ($F_{14, 135} = 9.103$; $P < 0.001$). The cocoon width also showed a similar trend ($F_{14, 135} = 3.696$; $P < 0.001$). However, a non-significant difference was noted in the cocoon length of the experimental group as compared to control ($F_{14, 135} = 1.456$; $P > 0.05$). The silkworms fed with 1 % Alanine treated mulberry leaves showed the maximum cocoon weight (1.35 g ± 0.009), length (30.29 mm ± 0.578) and width (14.90 mm ± 0.310) (Table 2).

3.3. Cocoon shell ratio

The cocoon shell ratio in all treatment groups was higher than the control group. However, the cocoon shell ratio of the group treated with 1 % Alanine was significantly higher than the control ($F_{14, 135} = 47.317$; $P < 0.001$) followed by 1 % Glycine. The cocoon shell ratio was highest in silkworms fed with 1 % Alanine treated mulberry leaves (35.21 ± 0.361) followed by silkworms fed with 1 % Glycine treated mulberry leaves (32.37 ± 0.559) (Table 3).

3.4. Estimation of % fibroin and % sericin content

The percentage fibroin content of experimental groups treated with 1 % Alanine was significantly higher followed by 1 % Glycine as compared to control ($F_{14, 30} = 14.017$; $P < 0.001$); however, all other groups also showed a statistically significant difference from the control. Treatment groups in which silkworms were fed with 1 % Alanine treated mulberry leaves followed by 1 % Glycine treated

Table 2
Increase in larval weight (g) in control and experimental groups.

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	$0.054^{a} \pm 0.002$	$0.157^{a} \pm 0.008$	$0.234^{a} \pm 0.008$	$0.295^{a} \pm 0.009$	$0.337^{a} \pm 0.011$	$0.373^{a} \pm 0.013$
1 % A	$0.131^{c} \pm 0.014$	$0.299^{d} \pm 0.013$	$0.422^{e} \pm 0.013$	$0.520^{e} \pm 0.013$	$0.599^{e} \pm 0.013$	$0.665^{e} \pm 0.013$
1 % G	$0.095^{bc} \pm 0.011$	$0.249^{bc} \pm 0.007$	$0.325^{bc} \pm 0.012$	$0.399^{bc} \pm 0.012$	$0.461^{bcd} \pm 0.014$	$0.527^{bcd} \pm 0.016$
1 % S	$0.106^{bc} \pm 0.014$	$0.259^{bcd} \pm 0.005$	$0.347^{bcd} \pm 0.010$	$0.437^{bcd} \pm 0.016$	$0.498^{cd} \pm 0.016$	$0.545^{cd} \pm 0.017$
1 % A + G	$0.101^{bc} \pm 0.011$	$0.259^{bcd} \pm 0.007$	$0.335^{bcd} \pm 0.008$	$0.421^{bcd} \pm 0.010$	$0.489^{bcd} \pm 0.011$	$0.544^{cd} \pm 0.013$
1 % A + S	$0.083^{ab} \pm 0.005$	$0.271^{cd} \pm 0.009$	$0.341^{bcd} \pm 0.010$	$0.428^{bcd} \pm 0.015$	$0.469^{bcd} \pm 0.016$	$0.507^{bcd} \pm 0.016$
1 % G + S	$0.085^{ab} \pm 0.008$	$0.252^{bcd} \pm 0.015$	$0.324^{bc} \pm 0.016$	$0.402^{bc} \pm 0.019$	$0.448^{bc} \pm 0.018$	$0.501^{bcd} \pm 0.016$
1 % A + G + S	$0.162^{ab} \pm 0.006$	$0.278^{cd} \pm 0.008$	$0.340^{bcd} \pm 0.007$	$0.398^{bcd} \pm 0.011$	$0.472^{cd} \pm 0.012$	$0.523^{cd} \pm 0.015$
0.5 % A	$0.104^{bc} \pm 0.010$	$0.267^{cd} \pm 0.007$	$0.332^{bc} \pm 0.010$	$0.409^{bcd} \pm 0.014$	$0.454^{bc} \pm 0.013$	$0.493^{bc} \pm 0.013$
0.5 % G	$0.077^{ab} \pm 0.003$	$0.233^{bc} \pm 0.011$	$0.353^{cd} \pm 0.015$	$0.429^{bcd} \pm 0.016$	$0.467^{bcd} \pm 0.017$	$0.497^{bcd} \pm 0.018$
0.5 % S	$0.083^{ab} \pm 0.005$	$0.245^{bc} \pm 0.010$	$0.355^{cd} \pm 0.017$	$0.432^{bcd} \pm 0.017$	$0.474^{bcd} \pm 0.017$	$0.516^{bcd} \pm 0.015$
0.5 % A + G	$0.075^{ab} \pm 0.003$	$0.260^{bcd} \pm 0.008$	$0.397^{de} \pm 0.013$	$0.480^{de} \pm 0.015$	$0.531^{de} \pm 0.015$	$0.572^{d} \pm 0.015$
0.5 % A + S	$0.081^{ab} \pm 0.004$	$0.254^{bcd} \pm 0.008$	$0.363^{cde} \pm 0.015$	$0.461^{cde} \pm 0.020$	$0.520^{cd} \pm 0.020$	$0.566^{cd} \pm 0.020$
0.5 % G + S	$0.079^{ab} \pm 0.003$	$0.252^{bcd} \pm 0.008$	$0.360^{cde} \pm 0.012$	$0.448^{bcde} \pm 0.013$	$0.502^{cd} \pm 0.014$	$0.543^{cd} \pm 0.014$
0.5 % A + G + S	$0.070^{ab} \pm 0.004$	$0.213^{b} \pm 0.013$	$0.291^{ab} \pm 0.014$	$0.379^{b} \pm 0.017$	$0.414^{b} \pm 0.017$	$0.451^{b} \pm 0.016$

Note. Values in column with similar superscripts showed non-significant difference while values with different superscripts showed significant difference. The values after ± are representing Standard Error.

Table 3
Comparison of Economic traits in experimental groups and control group.

Groups	Cocoon Weight	Cocoon Length	Cocoon Width	% Shell Ratio	% Fibroin Content	% Sericin Content
Control	$0.983^{a} \pm 0.015$	$26.97^{a} \pm 0.571$	$12.93^{a} \pm 0.377$	$21.55^{a} \pm 0.566$	$70.13^{a} \pm 0.954$	$29.87^{e} \pm 0.953$
1 % A	$1.313^{e} \pm 0.041$	$30.29^{a} \pm 0.578$	$14.90^{b} \pm 0.310$	$35.21^{h} \pm 0.361$	$85.35^{e} \pm 0.733$	$14.65^{a} \pm 0.736$
1 % G	$1.293^{de} \pm 0.034$	$29.98^{a} \pm 0.616$	$13.81^{ab} \pm 0.316$	$32.37^{g} \pm 0.559$	$84.15^{de} \pm 0.866$	$15.85^{ab} \pm 0.866$
1 % S	$1.158^{bcd} \pm 0.024$	$28.29^{a} \pm 0.505$	$13.05^{a} \pm 0.178$	$29.35^{f} \pm 0.558$	$79.32^{cd} \pm 1.341$	$20.68^{bc} \pm 1.341$
1 % A + G	$1.099^{abc} \pm 0.030$	$27.57^{a} \pm 0.604$	$13.09^{a} \pm 0.192$	$28.14^{f} \pm 0.240$	$75.82^{abc} \pm 0.343$	$24.18^{cde} \pm 0.343$
1 % A + S	$1.096^{ab} \pm 0.024$	$27.31^{a} \pm 0.769$	$13.14^{a} \pm 0.213$	$27.89^{ef} \pm 0.986$	$73.69^{abc} \pm 1.5$	$26.31^{cde} \pm 1.5$
1 % G + S	$1.171^{bcd} \pm 0.015$	$27.40^{a} \pm 0.875$	$13.01^{a} \pm 0.235$	$23.46^{abc} \pm 0.695$	$77.90^{bc} \pm 0.275$	$22.10^{cd} \pm 0.275$
1 % A + G + S	$1.108^{abc} \pm 0.028$	$27.45^{a} \pm 1.253$	$13.22^{a} \pm 0.342$	$24.40^{bcd} \pm 0.593$	$74.99^{abc} \pm 0.927$	$25.01^{cde} \pm 0.927$
0.5 % A	$1.206^{bcde} \pm 0.029$	$27.36^{a} \pm 0.692$	$13.05^{a} \pm 0.189$	$26.71^{def} \pm 0.663$	$72.87^{ab} \pm 0.525$	$27.13^{de} \pm 0.525$
0.5 % G	$1.214^{bcde} \pm 0.033$	$27.98^{a} \pm 0.832$	$12.99^{a} \pm 0.227$	$25.32^{cde} \pm 0.494$	$75.86^{abc} \pm 0.480$	$24.14^{cde} \pm 0.480$
0.5 % S	$1.258^{de} \pm 0.012$	$27.66^{a} \pm 0.775$	$13.35^{a} \pm 0.154$	$23.49^{abc} \pm 0.043$	$78.80^{cd} \pm 0.590$	$21.20^{bc} \pm 0.590$
0.5 % A + G	$1.222^{bcde} \pm 0.029$	$27.67^{a} \pm 0.579$	$13.12^{a} \pm 0.165$	$23.16^{abc} \pm 0.240$	$72.90^{ab} \pm 0.368$	$27.10^{de} \pm 0.368$
0.5 % A + S	$1.238^{cde} \pm 0.035$	$27.67^{a} \pm 0.791$	$13.31^{a} \pm 0.222$	$24.91^{bcd} \pm 0.468$	$76.15^{bc} \pm 1.973$	$23.85^{cd} \pm 1.973$
0.5 % G + S	$1.229^{bcde} \pm 0.019$	$27.68^{a} \pm 0.736$	$13.03^{a} \pm 0.243$	$22.34^{ab} \pm 0.514$	$76.25^{bc} \pm 0.511$	$23.75^{cd} \pm 0.511$
0.5 % A + G + S	$1.229^{bcde} \pm 0.025$	$27.68^{a} \pm 0.644$	$13.16^{a} \pm 0.181$	$22.56^{abc} \pm 0.471$	$72.87^{ab} \pm 0.743$	$27.13^{de} \pm 0.743$

Note. Values in column with similar superscripts showed non-significant difference while values with different superscripts showed significant differences. The values after ± are representing Standard Error.

mulberry leaves showed the highest percentage fibroin content (85.35 ± 0.733 and 84.15 ± 0.866) respectively as compared to control (70.13 ± 0.954) and other twelve treatment groups (Table 3). There was a significant difference in the amount of sericin in control and experimental groups ($F_{14, 30} = 14.018$; $P < 0.001$), the highest percentage in control (29.87 ± 0.953) while the lowest in the group treated with 1 % Alanine fortified mulberry leaves (Table 3).

4. Discussion

The growth and development of silkworms (*Bombyx mori* L.) can be improved by using supplemented nutrition which in return affects the overall quantity and quality of silk cocoon produced [41]. In this study, the commercial and biological traits of *B. mori* were observed after feeding them with amino acids fortified mulberry leaves. The silkworms have an equal tendency to consume both the fortified and non-fortified mulberry leaves [37]. Our study showed that the silkworm larvae fed with mulberry leaves supplemented with amino acids showed a significant improvement in both biological and commercial traits as compared to the control. Similar results were also reported in silkworm larvae when fed with vitamin and dietary proteins supplemented diet [42,43].

Naturally, the weight of silkworm larvae increases gradually during their life cycle. In our study, the larval weights as well as the percentage ratio of silk gland weight to the body weight of larvae fed with amino acid fortified mulberry leaves were significantly higher as compared to larvae of the control group at day 1–7 of 5th instar. A similar increase in larval weight and silk gland weight was also reported in silkworm larvae when they were fed with mulberry leaves supplemented with raw whey protein, royal jelly and egg white [37,44]. The highest weight of larvae was recorded in silkworms fed with 1 % alanine coated mulberry leaves. Similarly, the maximum increase in the percentage ratio of silk gland to body weight was observed in larvae fed with 1 % Alanine fortified mulberry leaves. Radjabi (2010) also recorded a similar increase in the silkworm larvae weight against 5 % alanine and 5 % glycine enriched mulberry leaves [45]. Devi and Yellamma (2013) also reported the gradual increase of both the silkworm larvae weight and percentage ratio of silk glands, when fed on Pyridoxine, Zinc chloride and Methoprene but these supplements are costly and not easily available [46]. Therefore, in the current study amino acid fortified mulberry leaves were used as they are cost-effective.

The possible reason for these results could be the fact that the actual silk fiber is mainly composed of three amino acids *i.e.,* Alanine, Glycine and Serine. The use of these amino acids as a food supplement in the diet of silkworms could be the possible reason for enhanced growth and better silk cocoon parameters. Alanine plays a vital role in the metabolism of organic acid, glucose and tryptophan [47]. It also boosts immunity and nourishes the brain, muscles, and central nervous system as well as plays a key role in the development of insect exoskeleton [48]. Glycine and serine are involved in the regulation of cross links between the fat body and silk gland. Furthermore, these amino acids can also regulate nutrient absorption and inhibitory neurotransmitter signaling [7].

As the biological growth of silkworm larvae is linked with its commercial traits, the effect of amino acid supplemented mulberry leaves against six different commercial traits of silkworms were also assessed. The results of our study showed that mulberry leaves fortified with Glycine, Alanine and Serine have the potential to significantly increase the commercial traits of silkworms including cocoon weight, cocoon length, cocoon width, percentage shell ratio and percentage fibroin content as compared to control. 1 % alanine showed better results than other concentrations because the fortification of alanine at a particular level may possibly be effective in improving silkworm growth and enhancing its cocoon commercial parameters but due to its non-essential nature, a higher level of alanine fortification may not have a positive effect on the biological and economic parameters of silkworm [49]. It exhibited its beneficial impact than other amino acids because it enhances organic acid and glucose metabolism [47]. Our results are in accordance with Murugesh et al. (2022), who also reported the improvement in biological traits and cocoon parameters in larvae fed with Glycine, Alanine and Serine fortified mulberry leaves respectively, but in Bivoltine Double Hybrid silkworm [11].

However, there are also a few limitations associated with the practical adoption of silkworm feed supplements described in the present study. It is challenging to convince silkworm farmers, so that they may add amino acid supplements to the silkworm's feed.

Furthermore, it is important to use amino acids supplements at specific concentrations, as higher concentrations can lead to toxicity. Here it is concluded that amino acid fortification of silkworm (*Bombyx mori* L.) feed has great potential in the sericulture industry and if applied properly it can lead to the enhancement of overall productivity of this cottage based industry.

Data availability statement

Here it is stated that the data associated with this study is not deposited into a public repository and it will be made available on demand.

CRediT authorship contribution statement

Ayesha Muzamil: Conceptualization, Methodology, Project administration, Writing – original draft. **Hafiz Muhammad Tahir:** Conceptualization, Methodology, Supervision, Validation. **Aamir Ali:** Project administration, Writing – original draft, Writing – review & editing. **Muhammad Farooq Bhatti:** Formal analysis, Methodology, Project administration, Resources, Software. **Fariha Munir:** Conceptualization, Data curation, Formal analysis, Software, Validation. **Fatima Ijaz:** Investigation, Methodology, Project administration, Visualization, Writing – review & editing. **Muhammad Adnan:** Validation, Writing – original draft, Writing – review & editing. **Hooria Ashraf Khan:** Formal analysis, Investigation, Methodology, Project administration, Validation. **Kunza Abdul Qayyum:** Methodology, Project administration, Resources, Writing – original draft.

Declaration of generative AI and AI-assisted technologies in the writing process

Here it is stated that no AI and AI-assisted technologies were used during the writing of this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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